Assay Solution
A Solution Provider

iPCR Conversion Kit

Catalog #: IPA-10100

User Manual

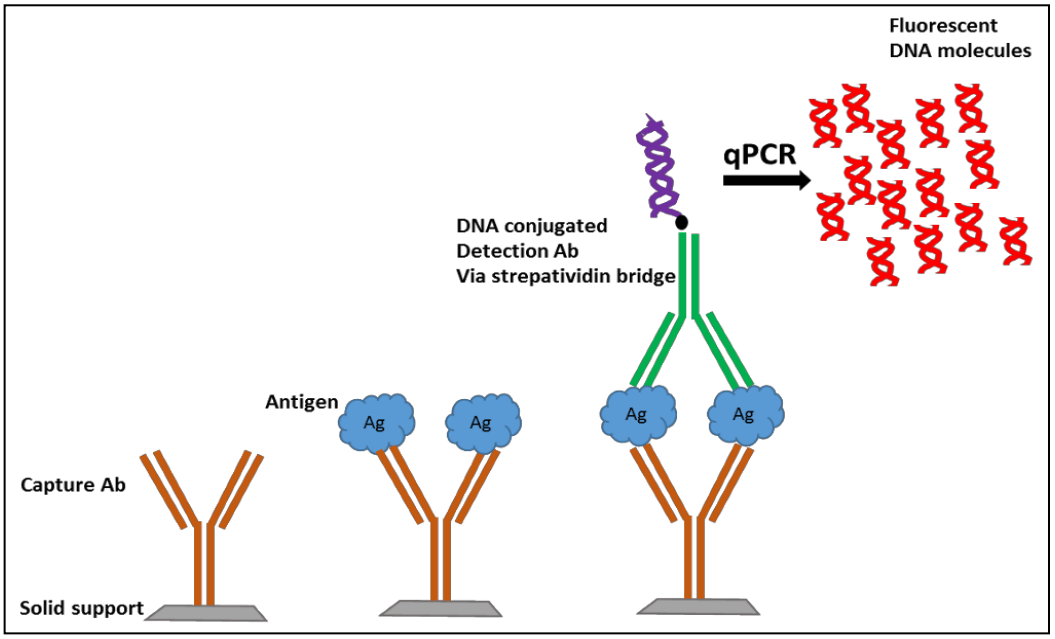
This kit is designed to convert an ELISA to highly sensitive immune PCR.

Manufactured and Distributed by:
Assay Solution
310 W Cummings Park STE A, Woburn, MA, 01801, USA
Phone: (617) 575-7215, Fax: (617) 380-0053
Email: Info@assaysolution.com

Description

Immuno-PCR (iPCR) is a powerful method for detecting ultra-low quantities of antigens. It combines the advantages of both enzyme-linked immunosorbent assay (ELISA) and PCR in specificity, sensitivity and ease to adapt. Despite its potential, iPCR has been an underutilized method as evidenced by the low number of publications on its routine application and unavailability of validated, ready-to-use commercial kits. Situation changed in recent years since more and more evidence indicates iPCR is a very helpful tool to improve detection sensitivity. To make it possible for the researchers to detect various low-abundant analytes, AssaySolution developed a validated ready-to-use iPCR conversion kit.

Assay principle



Materials Supplied

Component	Amount
Biotinylated DNA (100X)	1 vial, 40 µl
Streptavidin (100X)	1 vial, 40 µl
Primer mix (with taqman probe) 100X	1 vial, 40 µl
PCR Master mix (2X)	1 vial, 1.5 mL
Wash buffer (20X)	25 ml
Blocking buffer (10X)	12 ml
Buffer B (10X)	25 ml
TopYield Strip and frame	1 set
qPCR plate Sealer	2

Materials required but not supplied

1. Capture antibody and biotinylated detection antibody.
2. Antigen standards.
3. High binding ELISA plate combined with a qPCR plate (for procedures with DNA release by BamHI digestion).
4. 1X TBS
5. 1X PBS
6. Nuclease-Free water

Reagent Preparation

- Dilute the supplied **10X Blocking Buffer** to 1X Blocking Buffer using 1X TBS.
- Dilute the supplied **10X Buffer B** to 1X Buffer B using 1X TBS.
- Dilute the supplied **100X Streptavidin** (purple cap) to 1X using 1X Blocking Buffer.
- Dilute the supplied **100X Biotinylated DNA** (blue cap) to 1X using Nuclease-Free water.
- Dilute the supplied **20X Wash Buffer** to 1X Wash buffer using water.
- Prepare the 1X PCR mix by diluting the supplied **2X PCR Master mix** (clear cap) and **100X Primer mix** (red cap) to 1X final concentration using water.

Assay Procedure

1. Dilute the capture antibody (0.2 – 5 µg/ml) in PBS and add 30 µl to each Topyield plate well. Seal the plates with the adhesive cover and incubate overnight at 4°C (or as recommended by the manufacturer).
2. Wash the plate 3 times with 1X Wash buffer, each time incubating for 1 min at room temperature.
3. Add 300 µl 1X Blocking buffer. Seal with an adhesive cover and incubate at 4°C overnight.
4. Wash the plate 3 times with 1X Wash buffer, each time incubating for 1 min at room temperature.
5. Serially dilute the antigen standards (1:10) in 1X Blocking buffer and add 30 µl in each well. Add samples to designated wells with user-defined dilutions.
6. Incubate at room temperature for 30 min - 1 hour.
7. Wash the plate 3 times with 1X Wash buffer, each time incubating for 1 min at room temperature.
8. Dilute the biotinylated detection antibody in 1X Blocking buffer (0.1 – 2 µg/ml) and add 30 µl in each well.
9. Incubate at room temperature for 45 minutes.
10. Wash the plate 3 times with 1X Wash buffer, each time incubating for 1 min at room temperature.
11. Add 30 µl of 1X Streptavidin to each well.
12. Incubate at room temperature for 45 minutes.
13. Add 30 µl of 1X Biotinylated DNA to each well and incubate at room temperature for 45 minutes.
14. Wash the plate with 1X Buffer B 7 times, incubating 5 min during each wash step.
15. Wash with PBS twice incubating 1 min during each wash step.

Optional:

The assay sensitivity could be increased by digesting the biotinylated DNA with BamH1 after step 15 in Assay procedure (one BamH1 restriction site is included in the biotinylated DNA close to the streptavidin binding terminal). Treat each well with 30 µl BamH1 in its reaction buffer and incubate at 37°C for 1-2 hours. Transfer 2-5 µl of the above digested DNA into a 96-well qPCR plate and perform the reaction (final volume 20-30 µl) as mentioned in the step 16.

16. Add 30 µl of 1X PCR mix to each well. Seal with a PCR film and transfer to PCR cycler with an optical compression pad.

Carry out the program as mentioned below:

Time	Temperature	Cycles
5 min	95 °C	1X
30 s	50°C	28X
30 s	72 °C	
12 s	95 °C	

Note: Set up FAM (emission at 518 nm) as the fluorophor and tetramethylrhodamine as the quencher.

Results and Data Analysis

1. Calculate threshold cycles (Ct). The instrument automatically calculates the threshold cycle (Ct), which represents the first PCR cycle at which the fluorescent reporter signal (dR) exceeds the signal of a given uniform 'threshold', manually set it in the phase where signal increases linearly (typically 100–3,000). Use a half-logarithmic plot of log dR against cycle number to choose the correct threshold value. Alternatively, use the threshold suggested by the instrument software.
2. Export the Ct signals to an appropriate program for further data analysis (such as Microsoft Excel).

3. Calculate dCt signals by subtraction of the maximal number of cycles (CMax) and the Ct value (e.g., for CMax = 40, dCt = 40–Ct).

4. For each standard and unknown sample analyzed in duplicate, calculate mean values and s.d. of dCt.

5. For quantification, perform either a linear regression of the appropriate detection range of the calibration curve CC1–CC7 (plot the dCt signals against the log spiked concentration for linear correlation) or use a nonlinear regression (e.g., 4-point fit) for the complete calibration curve. If a specific detection window is analyzed in several assays, the calibration curve should be adapted for linear correlation between signals and log concentration for all seven CC samples. The resulting equation is used for the quantification of unknown samples and the calculation of recovery rates of spiked samples.

   Example assay:

Protein concentration (pg/mL)	Ct
1000	17.87
100	21.76
10	26.59
1	30.08
0.1	33.35
0.001	37.64